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TECHNICAL MANUSCRIPT 613

LOSS OF PICLORAM AND 2,4,5-T  
FROM THE ROOTS  
OF ASH AND MAPLE SEEDLINGS

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MAY 1970

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LOSS OF PICLORAM AND 2,4,5-T FROM THE ROOTS  
OF ASH AND MAPLE SEEDLINGS

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Project 1B562602AD04

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### ABSTRACT

Sublethal concentrations of  $C^{14}$ -picloram (4-amino-3,5,6-trichloropicolinic acid) and  $C^{14}$ -2,4,5-T (2,4,5-trichlorophenoxyacetic acid) were foliarly applied to red maple and green ash seedlings grown in nutrient solution.  $C^{14}$ -picloram was also applied to the leaves of white ash.

Radioactivity was detected in nutrient solution samples from all treated plants within 24 hours.  $C^{14}$ -picloram loss through the roots increased over a 3-week period, with the exception of a slight decrease in the amount lost on the 6th and 16th day from the green ash. The total radioactivity in the white ash nutrient solution increased over the 9-day experiment.

The nutrient solution samples of the red maple treated with  $C^{14}$ -2,4,5-T yielded radioactivity within 24 hours; this amount increased over the 3-week sampling period except for a decrease on the 16th day. The green ash nutrient solution showed  $C^{14}$ -2,4,5-T activity at 48 hours and increased throughout the 3 weeks. Greater loss of both labeled herbicides occurred from red maple than from green ash.

The fact that  $C^{14}$  activity in the exudates was associated with unaltered herbicide molecules was substantiated by bioassays.

## I. INTRODUCTION\*

Ash (Fraxinus spp.) has been reported to be generally resistant to picloram, although red maple (Acer rubrum) is susceptible. Both ash and maple are also reported to be relatively tolerant to 2,4,5-T.<sup>1-3</sup> These differences in the responses of ash and maple to picloram and 2,4,5-T have not been completely explained, although attempts to explain the differences have been made through studies of phloem and xylem transport systems and foliar uptake mechanisms.<sup>1,3,4</sup>

Root excretion as a means of detoxifying foliarly applied herbicides has been suggested,<sup>5</sup> and numerous reports state that both exogenous and endogenous substances are root-excreted by a wide range of plants.<sup>6-9</sup> In view of this and the fact that picloram has been reported to be excreted from the roots of bean,<sup>10,11</sup> it was considered worthwhile to study the possibility that ash and maple tolerance to picloram or 2,4,5-T is related to root excretion. Therefore, the goal of these experiments was twofold, to determine (i) if picloram and 2,4,5-T are root-excreted by ash and maple, and (ii) if so, are they in sufficient quantities to explain differential tolerance to the two herbicides.

## II. MATERIALS AND METHODS

Uniform seedlings of red maple (Acer rubrum L.), green ash (Fraxinus pennsylvanica Marsh.), and white ash (Fraxinus americana L.) were maintained in containers of 0.5X Hoagland's solution under controlled environmental conditions in a growth chamber.

After one day in solution culture, sublethal dosages of C<sup>14</sup>-labeled picloram (4.25 µc/mg) or C<sup>14</sup>-labeled 2,4,5-T (4.25 µc/mg) in 95% ethanol were applied to the two leaves at the fourth whorl above the root collar. Each plant received ten 5-µl droplets of the labeled herbicide, which were applied within lanolin rings on the upper surface of the leaf with a calibrated microliter syringe.

The red maple and green ash seedlings were treated with both labeled herbicides; the white ash seedlings received only labeled picloram. The containers were brought up to a predetermined volume before 5-ml samples were taken daily for 7 days and then every 2 to 3 days until the termination of the red maple and green ash studies at 22 days. The white ash experiment covered a 9-day period; samples were taken each day.

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The samples of the nutrient solution were evaporated to dryness under vacuum and counted in a liquid scintillation counter to determine the amount of  $C^{14}$  present. The background count was determined from nutrient solution samples of untreated plants.

Bioassays were conducted to determine if the areas of biological activity and radioactivity in the nutrient solution were in agreement. In order to do this, the nutrient solution was filtered and evaporated to a 435-fold decrease in volume. The solution was then spotted on Whatman No. 20 chromatograph paper and co-chromatographed with labeled picloram and 2,4,5-T standards. Both the biological activity and radioactivity of the developed chromatographs were determined, the former by a lettuce seed bioassay<sup>12</sup> and the latter by a strip-scanner.

### III. RESULTS

The radioactivity of the mean cumulative total of  $C^{14}$  for replicates at each sampling period for the red maple and green ash is shown in Figure 1.

Loss of picloram was observed within 24 hours in both red maple and green ash. The amount of picloram lost increased over the 22-day period, except for slight decreases on the 6th and 16th days of sampling.

The maple nutrient solution showed the presence of labeled 2,4,5-T within 24 hours, and the amount increased over the entire sampling period except for a decrease on the 16th day. Loss of 2,4,5-T was evident in the ash nutrient solution within 48 hours, and increased over the 3 weeks of the experiment.

A greater loss of both herbicides was noted in the nutrient solution samples of the red maple seedlings compared with those from the green ash. The decrease in amount of radioactivity in samples taken on the 16th day has not been satisfactorily explained.

The cumulative total  $C^{14}$  activity of the nutrient solution samples of each replicate of the white ash seedlings is shown in Figure 2. The amount of  $C^{14}$  lost by the plant increased over the 9-day treatment period. There was considerable variability in the quantity of  $C^{14}$  lost by the replicates, but the maxima and minima generally are in agreement for all of the replicates on a given sampling. The 19,200 counts per minute (cpm) on day 9 represents 4.3% of the total picloram applied.

The lettuce seed bioassays of the nutrient solution showed that biological activity indicating the presence of picloram closely coincided with the areas of radioactivity when the nutrient solution was co-chromatographed with picloram and 2,4,5-T standards.

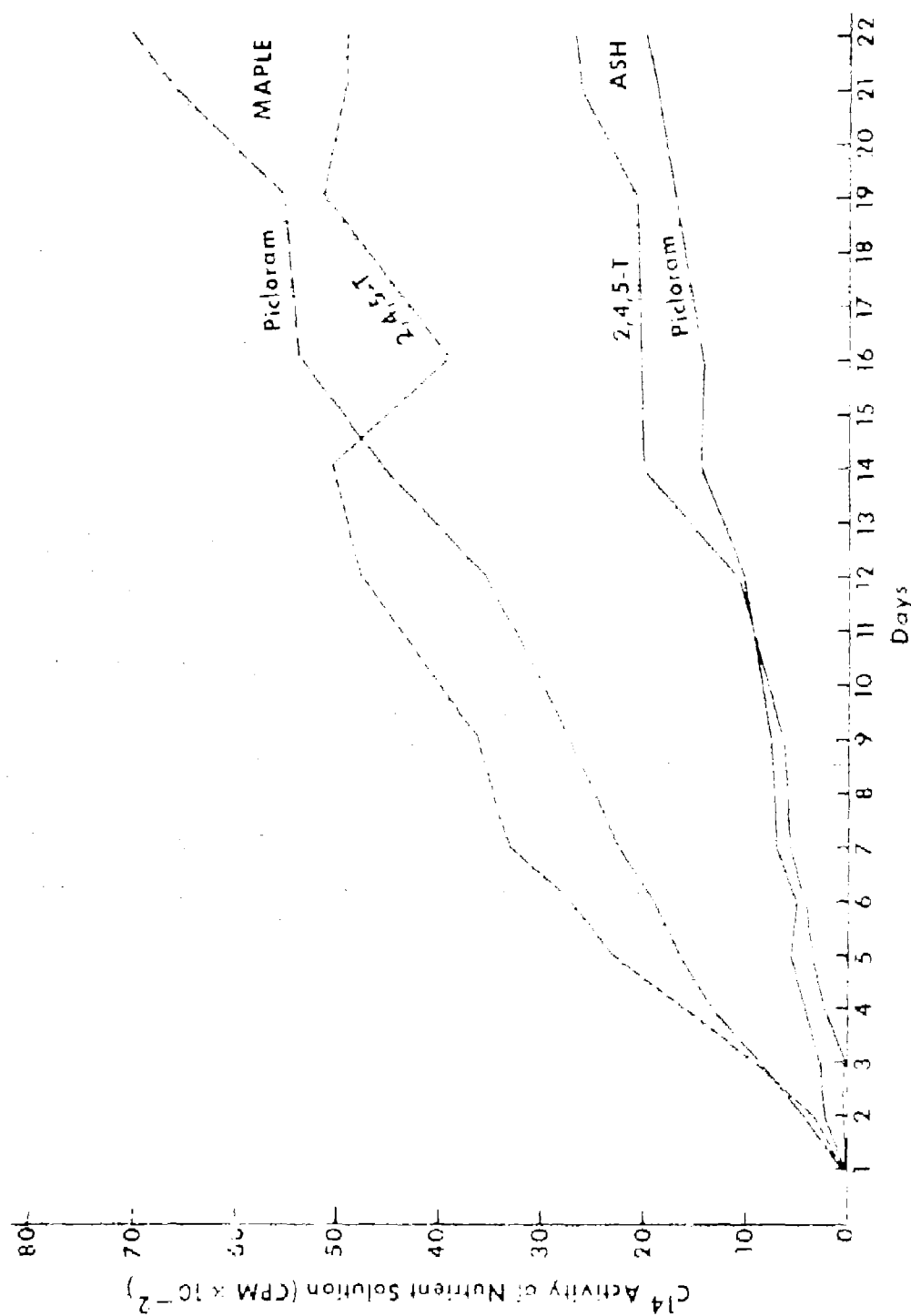


FIGURE 1. Cumulative  $^{14}\text{C}$  Loss from Roots of Red Maple and Green Ash after Foliar Treatment with 0.1  $\mu\text{g}$  (24  $\mu\text{g}$ ) of  $^{16}\text{C}$ -Picloram and  $^{14}\text{C}$ -2,4,5-T. Each plotted line represents the mean of six replicates.



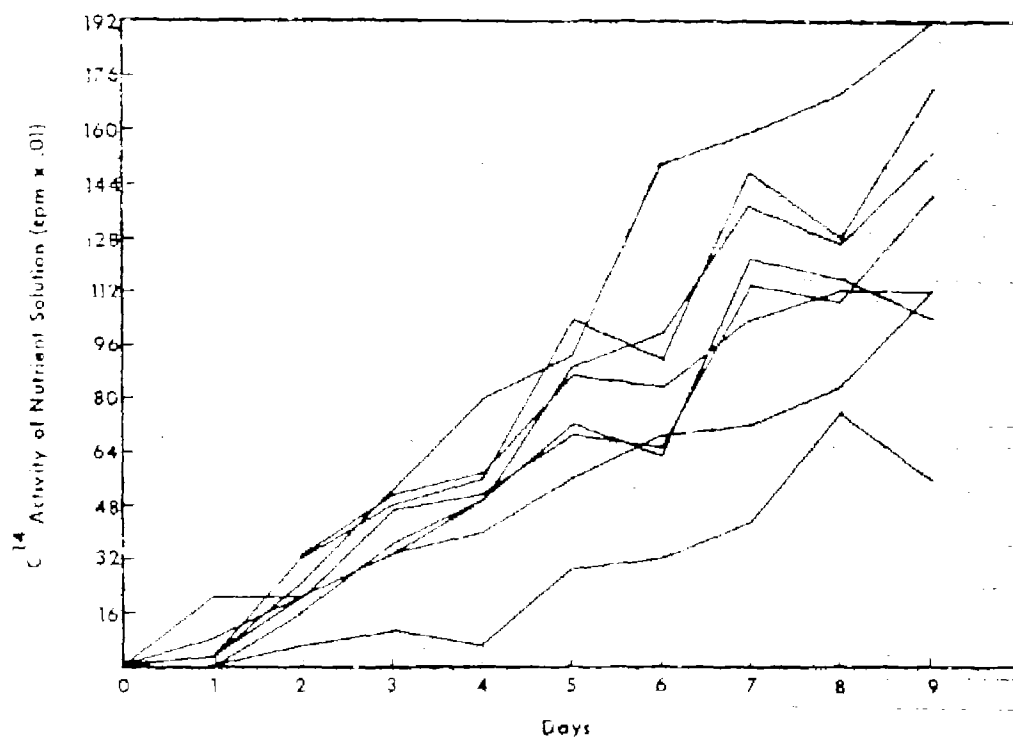


FIGURE 2. Cumulative  $C^{14}$  Loss from Roots of White Ash after Foliar Treatment with 0.1  $\mu$ Ci (24  $\mu$ g) of  $C^{14}$ -Picloram. Each plotted line represents one replicate consisting of two trees in 500 ml of nutrient solution.

#### IV. DISCUSSION

Data from these tests show that significant quantities of picloram and 2,4,5-T are lost from the roots of red maple and of green and white ash seedlings. The amounts of herbicides lost from the roots appears to have no consistent relationship with species tolerance or resistance. This would suggest that root exudation, as a detoxification mechanism, does not determine species response to the particular herbicide.

The amounts of picloram and 2,4,5-T lost by the roots of these woody species may, however, be of considerable ecological importance. The effects of root-excreted substances on other organisms (allelopathy) has been recognized by others.<sup>9,13-15</sup> Although Mitchell and Linder<sup>9</sup> believed that root exudation of exogenous compounds is of no practical significance, they did acknowledge that the growth of subsequent crops or nearby plants might be affected by exuded substances that persist in the soil. The reported persistence of picloram in soils<sup>16,17</sup> may make its exudation by woody species a factor of considerable ecological importance.

Mitchell and Linder<sup>9</sup> state in their review article that regulating substances exuded from roots were confined to the  $\alpha$ -methoxyphenylacetic acids and chlorinated benzoic acids. Since the time of their article, the exudation of 2,4-D<sup>5,18</sup> and picloram<sup>10,11</sup> have been reported. Neither of these compounds or 2,4,5-T falls within the two-family classification reported by Mitchell and Linder.

It should be realized that many factors found in the environment of soil-grown plants may differ from those observed in nutrient solution culture. These would include variation in temperature, humidity, light conditions, and the presence of microbes that could cause bio-degradation, as well as a host of unknown variables. The importance of these factors is illustrated by the findings of Meikle et al,<sup>19</sup> who have found that the combination of living plant and soil was more active in decomposition of picloram than either the plant or soil alone.

Thus, although a difference in species response can be seen at the physiological level, i.e., greater loss of both herbicides by the red maple, it is difficult to correlate this with previously reported differences in species tolerance. However, the fact that detectable amounts of exogenous compounds may be lost to the rhizosphere from the roots of treated plants should be considered in order to obtain a better understanding of the physiological and possible ecological aspects of the use of growth-regulating compounds for woody plant control.

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<p>Sublethal concentrations of C<sup>14</sup>-picloram (4-amino-3,5,6-trichloro-picolinic acid) and C<sup>14</sup>-2,4,5-T (2,4,5-trichlorophenoxyacetic acid) were foliarly applied to red maple and green ash seedlings grown in nutrient solution. C<sup>14</sup>-picloram was also applied to the leaves of white ash.</p> <p>Radioactivity was detected in nutrient solution samples from all treated plants within 24 hours. C<sup>14</sup>-picloram loss through the roots increased over a 3-week period, with the exception of a slight decrease in the amount lost on the 6th and 16th day from the green ash. The total radioactivity in the white ash nutrient solution increased over the 9-day experiment.</p> <p>The nutrient solution samples of the red maple treated with C<sup>14</sup>-2,4,5-T yielded radioactivity within 24 hours; this amount increased over the 3-week sampling period except for a decrease on the 16th day. The green ash nutrient solution showed C<sup>14</sup>-2,4,5-T activity at 48 hours and increased throughout the 3 weeks. Greater loss of both labeled herbicides occurred from red maple than from green ash.</p> <p>The fact that C<sup>14</sup> activity in the exudates was associated with unaltered herbicide molecules was substantiated by bioassays. ( )</p>		
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